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Editorial

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The journal *Alytes* was founded at the Paris National Museum of Natural History in February 1982. From November 1982 to November 1989, it was published by the Société Batrachologique de France (S.B.F.), although since March 1984 it has been subtitled "International Journal of Batrachology", and opened, besides those in French, to papers in English.

On 11 November 1988, the General Assembly of the S.B.F. decided the foundation of the ISSCA (International Society for the Study and Conservation of Amphibians), the transfer to this new international society of the journal *Alytes*, and the opening of the latter also to papers in Spanish. Since, however, publication of volume 7 of *Alytes*, due for the year 1988, was late, it was decided to wait until completion of this publication to transfer the journal to the ISSCA. This was done only in November 1989, and volume 8 of the journal will now be published by the ISSCA.

In order to suppress the one-year delay in the publication schedule of *Alytes* inherited from the S.B.F., the ISSCA Board decided that volume 8 of *Alytes* will formally cover both years 1989 and 1990 : therefore, at the end of this volume, the journal should have recovered normal dates of publication.

All subscribers who have paid in 1989 for their subscription to volume 8 of *Alytes* have therefore no additional subscription to pay for 1990, and will receive all issues of this volume.

Alytes is therefore starting again on new, truly international, bases. We invite warmly all batrachologists worldwide to submit to us for publication papers in English, French or Spanish dealing with all aspects of the scientific study and conservation of amphibians. Although the journal has until now mostly published papers in the fields of amphibian systematics and biogeography, we want to make it quite clear that its scope is not limited to these topics, but is much wider, including all fields of *amphibian biology* as such (e.g. amphibian ecology, ethology, physiology, karyology, genetics, evolution, developmental biology and embryology, larval biology, conservation biology, etc.), and excluding only experimental works where amphibians are used merely as a "material" but without paying attention to these organisms as such.

The Editorial Board of *Alytes* has been expanded and now includes 13 members from 8 countries. Any paper submitted for publication in *Alytes* will be entrusted to one of these members, who will act as Corresponding Editor for this paper, and send it out for review to two referees at least before making the final decision.

The delay for publication of an accepted paper in *Alytes* is currently of 3 to 6 months after submission, which is much less than in many other international journals of zoology or herpetology. No page charges are requested from authors, but we regret to have to charge the latter for the publication of color photographs.

We invite you all to join the ISSCA and to participate in the life and development of its journal *Alytes*, the first journal of batrachology in the world.

Images d'Amphibiens camerounais. I. Sacs vocaux et postures de chant

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Eight photos of anurans from Cameroon, taken during the emission of nuptial calls, are commented upon. These photos illustrate various aspects of the vocal sac and of the body during the call, as well as the postures taken by the frogs in relation to the shape of the vocal sac.

INTRODUCTION GÉNÉRALE

Sous le titre "Images d'Amphibiens camerounais" se succéderont de courts articles centrés sur des planches de photos en couleurs illustrant divers aspects de la biologie des Amphibiens Anoures du Cameroun, et plus spécialement leur reproduction.

Dans ces articles, conçus plutôt comme des "essais", le texte sera subordonné aux images et se limitera aux espèces de la batrachofaune camerounaise. Je laisse à d'autres le soin d'intégrer les informations ainsi apportées dans des travaux plus synthétiques. Dans la même perspective, seules seront citées les publications touchant directement aux thèmes traités.

Sur le plan technique, les documents photographiques présentés ne sont pas tous irréprochables. La plupart ont en effet été réalisés sur le terrain, dans des conditions parfois difficiles. Certains ont pâti de ma précipitation à fixer une scène rare ou fugace. J'espère que, tels quels, ces clichés parviendront quand même à faire partager aux lecteurs l'émotion que j'ai souvent ressentie en les réalisant.

REMERCIEMENTS

Ces articles n'auraient probablement jamais vu le jour sans les amicales pressions qu'ont exercées sur moi A. DUBOIS et J.-J. MORÈRE. Ils doivent aussi beaucoup aux suggestions et corrections de deux lecteurs à qui ils avaient été confiés : j'espère qu'ils se reconnaîtront dans les améliorations apportées au texte initial... tout comme je les ai reconnus à travers leurs critiques. A tous, j'exprime ma vive reconnaissance.

Aspects des sacs vocaux

Une des particularités remarquables de la phonation chez les Anoures est que les vocalisations (sauf les cris de détresse) sont produites la bouche fermée. Chez la plupart des espèces, il y a cependant une manifestation visible des émissions sonores, correspondant au gonflement d'un ou de deux sacs vocaux. Si l'on se réfère à l'une des synthèses récentes sur la question, celle de Duellman & Trueb (1986)[1], trois types de sacs vocaux peuvent être reconnus : subgulaire médian, subgulaire double et latéraux. A l'intérieur de chacune de ces catégories, en particulier la première, la forme, le volume, la coloration et la texture du tégument varient beaucoup suivant les genres et même les espèces considérés.

Chez les Anoures à sac médian subgulaire, le tégument de la gorge, dans le cas le plus simple, ne montre pas de différenciation particulière et a le même aspect dans les deux sexes. C'est ce qui s'observe, dans la faune camerounaise, chez les rainettes du genre *Leptopelis* ainsi que chez divers Bufonidae, Astylosterninae et Arthroleptinae.

Dans d'autres cas, le tégument gulaire a, chez le mâle, une coloration particulière, en général plus foncée. C'est ainsi que *Nyctibates corrugatus* (Astylosterninae) ne montre pas d'autre caractère sexuel secondaire que la coloration de la gorge, d'un noir d'encre chez le mâle (Amiet, 1973). Chez certaines espèces, le tégument gulaire des mâles peut aussi être plus ou moins spinuleux (*Petropedetes, Leptodactylodon*).

Ces diverses particularités de texture et de coloration n'ont pas de rapport évident avec l'émission des sons, contrairement aux plis gulaires, plus ou moins serrés et profonds, qui témoignent d'une plus grande extensibilité de la gorge lors du gonflement du sac vocal. De tels plis peuvent s'observer chez des *Leptodactylodon* (*albiventris* et le groupe d'*ovatus*) ou chez certains *Phrynobatrachus* (où ils sont, suivant les espèces, longitudinaux ou transverses).

Dans plusieurs genres de petites rainettes (*Hyperolius, Afrixalus, Kassina*...), cette différenciation du tégument gulaire s'accompagne de l'existence d'une zone glandulaire épaissie, de forme et de taille variables suivant les espèces. Cette structure a reçu diverses dénominations : "disque gulaire", "opercule", "protective flap", etc., auxquelles le terme de "glande gulaire" (Drewes, 1984) paraît préférable. La fonction de cette glande reste énigmatique (productions de substances stimulantes lors de l'accouplement, durant lequel le mâle appuie étroitement sa gorge sur la région occipitale de la femelle?) mais son rôle protecteur ne semble pas négligeable. Chez la plupart des espèces appartenant aux genres précités, la partie fine et vulnérable du tégument gulaire se replie en effet plus ou moins, au repos, sous le pourtour de la glande gulaire. Celle-ci, lorsque le sac vocal est en pleine extension, n'en représente qu'une faible surface, comme il est facile de le constater par les photos des fig. 1 à 3 (fig. 1 : *Hyperolius viridiflavus pallidus* ; fig. 2 : *Hyperolius ocellatus purpurescens* ; fig. 3 : *Kassina senegalensis*)[2].

Un perfectionnement remarquable de l'appareil vocal s'observe chez les grenouilles des genres *Ptychadena* et *Hildebrandtia* (voir fig. 5-6, *Hildebrandtia ornata*). Ici, comme chez

1. On pourra aussi consulter avec profit, sur ce même sujet, la mise au point de Paillette (1986).

2. On trouvera dans Drewes (1984) une analyse détaillée de la structure et de l'évolution du sac vocal chez les Hyperoliidae.



Fig. 1. – *Hyperolius viridiflavus pallidus* gonflant son sac vocal. Garoua, juillet 1975.

Fig. 2. – *Hyperolius ocellatus purpurescens* chantant sur une feuille. Yaoundé, novembre 1970.

Fig. 3 et 4. – *Kassina senegalensis*, même individu photographié sous deux angles différents, en phase de gonflement du sac vocal (3) et du corps (4). Minkama, octobre 1977.

d'autres Raninae, il y a deux sacs latéraux, mais qui peuvent s'invaginer ou faire saillie à l'extérieur en passant par deux fentes obliques situées sous les commissures buccales.

Dans la plupart des cas, l'émission d'un cri est précédée par le gonflement des poumons au-delà de leur capacité respiratoire normale. L'air ainsi accumulé est propulsé vers la cavité buccale et le sac vocal, faisant au passage vibrer les cordes vocales. L'air peut effectuer de la sorte plusieurs allers et retours successifs entre les poumons et le sac vocal.

Lors du remplissage des poumons, tout le corps du Batracien s'enfle de façon plus ou moins marquée. Ceci est rendu possible par la grande extensibilité du tégument : sur les photos des fig. 5 à 8, qui illustrent bien le gonflement alternatif du corps et du sac vocal, on remarquera que chez *Leptopelis brevirostris* (fig. 7-8), comme chez *Hildebrandtia ornata* (fig. 5-6), le tégument forme des plis sur les flancs lors de la phase de dégonflement.

Attitudes lors de la phonation

La possession d'un sac vocal, ou de deux, est un avantage pour la reproduction puisqu'elle permet l'amplification des appels nuptiaux produits par les mâles. Elle entraîne cependant – surtout chez les espèces à sac volumineux – quelques contraintes relatives au choix des postes de chant et au positionnement de l'animal lors de l'émission des appels.

En effet, chez les espèces à sac vocal subgulaire, le gonflement de celui-ci peut être entravé par son contact avec le substrat. Les fig. 7 et 8 montrent la curieuse posture qu'a dû adopter un mâle de *Leptopelis brevirostris* chantant sur une large feuille de Marantacée. Pour éviter que son sac vocal n'appuie sur le support, l'animal se soulève sur ses quatre membres, les postérieurs étant à demi fléchis, dans une attitude tout à fait inhabituelle chez un Anoure. Chez la *Kassina senegalensis* et l'*Hyperolius ocellatus* des fig. 2 et 3, on voit que le sac vocal a tendance à faire saillie vers l'avant plutôt que vers le bas et il suffit alors au chanteur de tendre ses seuls membres antérieurs.

Souvent, pour permettre le libre jeu du sac vocal, l'animal choisira de se tenir sur le bord d'une large feuille ou en travers d'une brindille : c'est le cas de l'*Hyperolius viridiflavus* de la fig. 1, dont le sac vocal ovoïde, à grand axe dorso-ventral, est presque aussi volumineux que l'animal lui-même.

Pour les espèces chantant sur le sol, les sacs latéraux paraissent représenter une solution pratique car l'animal n'est pas obligé de se camper sur ses membres antérieurs. Cette facilité ne joue guère pour *Hildebrandtia ornata* (fig. 5-6) car ses sacs vocaux sont si encombrants qu'elle ne peut pas chanter tapie sur le sol comme le font les *Ptychadena*.

Informations complémentaires sur les espèces figurées

Leptopelis brevirostris (Werner, 1898) (fig. 7-8)

Ce *Leptopelis* est largement répandu dans toute la zone forestière camerounaise, jusqu'à environ 1100 m d'altitude. C'est une espèce sylvicole qui, dans les régions à forte pluviosité, peut vivre aussi dans les formations secondaires denses. En revanche, il est peu fréquent en forêt mésophile et manque dans les forêts galeries les plus avancées.

L. brevirostris se distingue de ses congénères par deux particularités biologiques importantes :

– son régime alimentaire est essentiellement hélicophage, comme l'a montré PERRET (1966)[3] ;

– plusieurs indices montrent que son développement doit être direct : grande taille des ovules (5,2 mm de diamètre : PERRET, 1958), sites de chant des mâles souvent très éloignés de tout point d'eau (AMIET & SCHIØTZ, 1974), absence de Protozoaires endocommensaux dans le rectum (AMIET & AFFA'A, 1985).

HYPEROLIUS VIRIDIFLAVUS PALLIDUS MERTENS, 1940 (fig. 1)

Le nom de cette sous-espèce est bien choisi car la livrée dorsale peut devenir d'un blanc de craie. Au Cameroun, elle ne se rencontre que dans les plaines septentrionales, depuis le pied de l'Adamaoua jusqu'au Lac Tchad. A partir de la latitude de Poli, c'est le seul représentant du genre *Hyperolius* dans le Nord Cameroun.

HYPEROLIUS OCELLATUS PURPURESCENS LAURENT, 1943 (fig. 2)

C'est une espèce banale des endroits marécageux en forêt ou dans les formations secondaires denses juxta-forestières.

Le mâle figuré ici est en livrée nocturne, d'un roux ferrugineux, parfois rougeâtre, avec deux bandes jaune d'or latéro-dorsales et un triangle de même couleur sur le museau. En livrée diurne, l'animal est d'un vert translucide avec des bandes d'un jaune très pâle. La femelle a une livrée bien différente, ornée de petites taches claires circulaires pupillées de bleu.

KASSINA SENEGALENSIS (DUMÉRIL & BIBRON, 1841) (fig. 3-4)

Largement répandue dans les savanes et les steppes de l'Afrique sub-saharienne, *K. senegalensis* est peut-être en fait un amalgame de plusieurs espèces sub-jumelles.

Les mâles chantent souvent à plusieurs mètres de l'eau, dissimulés au pied des touffes d'herbe. Chez les *Kassina* (et les *Phlyctimantis*, qui en sont très proches), la glande gulaire est très développée et forme plus ou moins un "pont" reliant le bord antérieur de l'arc mandibulaire à la région pectorale. Une partie du tégument gulaire, très fine et pigmentée de noir, s'invagine de part et d'autre de la glande gulaire. Cette disposition pourrait laisser croire que, lors des émissions vocales, deux protubérances font saillie de chaque côté de la zone glandulaire. La fig. 3 montre que chez *K. senegalensis* il n'y a en fait qu'un volumineux sac vocal à contour régulièrement arrondi et orné de deux aires pigmentées de noir, ce qui avait déjà été observé par WAGER (1965)[4].

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3. Les informations données par PERRET sont ignorées par DREWES & ROTH (1981) dans leur travail sur deux autres Anoures hélicophages, *Tornierella kounhiensis* et *T. obscura*, mais ont été relevées par DUBOIS (1987 : 38).

4. En revanche, d'après le même auteur, chez *K. maculata* (autrefois appelée *Hylambates maculatus*) "two smaller balloons" apparaissent à la surface du sac vocal.

5



6

Fig. 5 et 6. – *Hildebrandtia ornata* en train de chanter, avec les sacs vocaux gonflés (6) et dégonflés (5). Mora, juillet 1973.

7



8

Fig. 7 et 8. — Deux phases de l'émission du chant chez *Leptopelis brevirostris*. Zamakoé, avril 1977.

Hildebrandtia ornata (Peters, 1878) (fig. 5-6)

Les *Hildebrandtia* sont une version fouisseuse des *Ptychadena*, avec lesquelles elles partagent de nombreux caractères, en particulier la possession de fentes sous-commissurales permettant la dévagination des sacs vocaux.

Localisées dans les savanes sèches et les steppes, ces grenouilles n'ont qu'une très brève période d'activité vocale en début de saison des pluies (Amiet, 1974) et les photos de mâles en train de chanter doivent être assez exceptionnelles.

Résumé

Huit photos d'Anoures camerounais, prises pendant l'émission des appels nuptiaux, sont commentées. Ces photos illustrent divers aspects du sac vocal et du corps lors du chant, ainsi que les postures adoptées en fonction de la forme du sac vocal.

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Tattooing as an individual marking technique in urodeles

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A review of the literature shows that no marking method of urodeles is entirely satisfactory. The choice of a method has to take into account the number of animals to be marked, the duration of the observations and the goals to be reached. Skin staining by tattooing is not often used even though such a technique gives interesting results. Tattooing is a quick procedure which makes it possible to mark a great number of animals individually. The remanence of the mark reaches at least three years. No harmful effects have been observed.

INTRODUCTION

Marking has always been a problem in studies of population dynamics in urodeles. No technique is entirely satisfactory and most often the solution chosen is a compromise.

Using rings, like bird bands, is made difficult because of the small size of urodeles limbs. Such rings involve a loss of mobility. We tried caudal rings derived from those applied to anuran limbs (DELY, 1954 ; NACE & MANDERS, 1982). Such rings, on which beads or symbols can be threaded, made it possible to study individual behaviour in the laboratory (MARTIN et al., 1989). But we sometimes observed newts trapped in a clump of vegetation, a blade of *Myriophyllum* wedged in the ring. After some time, necrosis may occur around the ring and may involve the loss of both a piece of the tail and the tag. The animal mobility would then be reduced until the lost part was regenerated.

Jaw tags used in anurans (RANEY, 1940) are too large for most urodeles and the risks of necrosis are the same as for caudal rings (RANEY, 1941).

Marking by branding avoids the need of carrying a foreign object and seems to be less traumatizing. Heat-branding using red-hot metal wire may make it possible to recognize a toad at least one year after marking (CLARK, 1971). But such a method has never been applied to urodeles. Freeze branding, using wires immerged in dry ice gives interesting results in fish (RICKER, 1956). In the anuran *Ascaphus truei*, scars remain readable for at least two years (DAUGHERTY, 1976). In urodeles however, freeze-branding scars don't persist longer than a few weeks (GEIGER et al., 1982, NACE & MANDERS, 1982, and personal observations) and may be traumatizing (KLEWEN, 1982). It is probable that such a regeneration capacity of the derm also rules out the heat branding technique.

Marking by toe-clipping is the technique which has been most frequently used for individual marking. Formalized by MARTOF (1953) in the study of a green frog population (*Rana clamitans*), such a method has been used in urodeles in the studies of TWITTY and his co-workers about the homing capacities of *Taricha rivularis* (synthesis in TWITTY, 1966). If it makes it possible to recognize a great number of individuals, it nevertheless has three major disadvantages :

(1) Urodele toes regenerate quickly : sometimes total regeneration requires less than one year. HEATWOLE (1961) suggested applying beryllium nitrate to inhibit regeneration. But, besides the use of such a highly toxic chemical is dangerous, its efficiency seems to be limited to temperatures below 18°C (EFFORD & MATTHIAS, 1969).

(2) The amputation of more than two digits involves a loss of mobility in anurans. The probability of recapturing a marked individual is inversely related to the number of clipped toes (CLARKE, 1972). The same observation is to be expected in urodeles.

(3) Newts often show natural digit amputation in the field ; this may be due to predatory insects like dragonfly larvae, particularly benthic *Libellula* larvae, or to small bivalves, like *Sphaerium*, which are often found gripping a toe.

Recognition of an individual thanks to its coloration pattern means organizing a collection of photographs. That efficient method may however only be used in species in which a pattern of spots varies from one individual to another, as on the backs of *Salamandra salamandra* (JOLY, 1968) and of *Notophthalmus viridescens* (HEALY, 1974) or on the belly of *Triturus cristatus*, *T. vulgaris* (HAGSTRÖM, 1973) and *T. boscai* (DIAZ-PANIAGUA, 1986).

In other cases, RAFINSKI (1974, 1977) recommended marking by autotransplantation of a piece of ventral skin on the back and *vice-versa*. An Alpine newt (*Triturus alpestris*) may support ten such grafts. Despite the advantage of giving permanent marks, that method has two major drawbacks :

(1) it concerns only species with contrasted body colours ;

(2) the time needed to perform several grafts and the limited number of combinations are handicaps for the study of numerous populations.

Lastly there are the skin staining techniques. For marking toads, WISNIEWSKI et al. (1980) and GITTINS et al. (1980) suggested the injection of alcyan blue into the limbs by use of a high speed anaesthetic injector, usually used by odontologists (Panjet or Dermojet). Using such an injector we tried to mark the belly skin of Alpine newts. The speed of projection was so strong that the jet of dye crossed the animal's body and came out on the other side, but without involving the animal's death. We estimated however the risk of organ injury to be too high to accept that technique for urodele marking.

The intradermic injection of a dye may be made possible by scarification or tattooing, as applied in anurans by KAPLAN (1958, 1959) or by using a spray gun (NISHIKAWA & SERVICE, 1988). Marks remain readable for at least two years and the entire marking procedure takes only a few minutes. The aim of our paper is to describe testing of such a technique in the newts *Triturus alpestris* and *T. helveticus*.

METHOD

TECHNIQUE

Its principle is to draw a pattern of spots on the animal's belly. Each spot is drawn by tattooing using an electric tattooer ; such a device is usually used by veterinarians for marking pets or farm animals. Alcyan blue and Indian ink provided colouring.

The newt is first anaesthetized by phenoxyethanol. Drawing a spot involves applying the tattooer for about 20 s. In order to obtain a 2 mm spot, the needles must be shifted slightly several times.

After rinsing the surplus dye (a paint brush is useful), the spot must be uniformly coloured and stand out well. The tracks of the tattooer needles have to be invisible. If the design is not correct, the procedure has to be repeated.

CHOSEN PATTERNS

Marking according to a site

That coding procedure makes it possible to estimate the newt flow between several sites. One spot indicates the site where the first capture occurred. If the newt is caught again in another site, a second spot is drawn according to a marking code. In the study of a *Triturus helveticus* population inhabiting four neighbouring ponds, each pond corresponded to a spot situated near a limb. Two spots meant that the animal had been caught at least twice in two different ponds, which were identified. Newt flows were thus estimated by a quick marking procedure and the organization of a complex file is unnecessary. But it does not provide information about individual behaviour.

Individual marking

It requires drawing a higher number of spots. In the case of a population of *Triturus alpestris*, we used a maximum pattern of 11 points. They were placed according to three longitudinal rows, which are situated on the right side, the middle and the left side of the belly. Each row can contain 5 spots. The middle row indicates the site of the first capture. It had only one spot. Lateral rows are used for individual recognition. If the newt moved from one site to another, no new spot is necessary, the animal retaining the individual code. In the data file, the individual code consists of three numbers :

- the first corresponds to the site of first capture, and ranges from 0 to 5 ;
- the second and the third correspond to right and left rows respectively ; they are composed of 0 to 5 figures, each ranging from 0 to 5.

Fig. 1 gives an example of the reading of such a code. Assuming the observer is able to recognize sex, such a coding system makes it possible to identify 2048 individuals. With only four spots per row, 512 newts may be recognized.

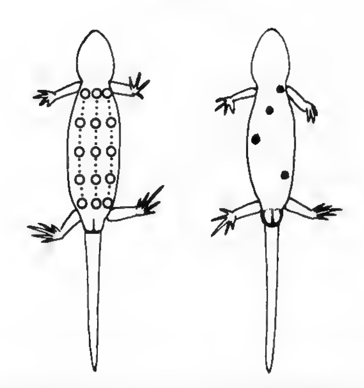


Fig. 1. – Marking patterns of newts. Left : overall view of the possible marking sites on the newt's belly : in the study of an Alpine newt population which was shared among 5 ponds, the two lateral rows were used for the individual marking whereas the middle row corresponded to the site of first capture. Right : example of a code : male newt n° 2/3/14 (a point at the second place on the middle row, a point at the third place on the right row, two points at the first and the fourth places on the left row).

Results

Permanence of the marks

In the laboratory, the suppression of hibernation, the high level of temperature (ranging from 16 to 25°C) and the abundance of food constitute conditions of frequent skin renewal. After two years under such conditions, marked Alpine newts could still be identified, despite a considerable decrease in the spot contrast. Under natural conditions, we suppose that the spot remains readable for at least 3 years.

Innocuousness

In the laboratory, none of the 4 marked newts died during the first year following the marking procedure. One of them died after one year. We can attribute such a death to natural mortality because the animals were relatively old when marked.

Table I. – Relation between number of spots and recapture probability.

Spots number	N newts marked in 1987	% recapture in 1988
2	68	19
3	140	12
4	41	22

Estimating the impact of marking on a wild population is more difficult. One possible way may be to compare the probabilities of recapturing a newt according to the number of spots drawn on its belly. If the method is harmful, the probability of recapturing should be related to the number of drawn spots. But no clear relation appears between percentages of recapture between two successive years and the number of spots (Table I).

DISCUSSION

Electric tattooing exhibits some interesting advantages :

- it makes the recognition of a great number of individuals possible;
- the marking lasts longer than that provided by toe-clipping;
- numerous animals may be treated quickly;
- the method seems innocuous.

However it does not provide the life-long permanence of the autotransplantation method. But the time needed for individual marking restricts the use of the latter method to small populations. The choice of one or the other (when recording of body pattern is not possible) depends on the kind of problem being dealt with and on the chosen experimental design.

Such a technique may be used in the field with a portable generator to provide electricity.

Because of their ventral position, the spots drawn on the belly are not apparent when the animal shows a normal posture. One may suppose that they do not interfere with the visual signals acting during courtship behaviour.

The use of such a marking technique is restricted to adult animals, and the problem of marking young newts in order to estimate juvenile dispersion is still unsolved.

The tattooing technique could be improved by trying new dyes which would last longer or would increase the number of combinations by diversifying the colours used.

Useful address : we used an electric tattooing device, which may be found at Veto-équipement , 188 avenue Roger Salengro, 69120, Vaulx-en-Velin, France.

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Studies on the biology of the tree-frog *Hyla arborea* during the breeding season in North Western Italy (Amphibia, Anura, Hylidae)

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Some parameters on the breeding phenology and larval period of *Hyla arborea* during 1988 and 1989 spawning seasons were investigated in six ditches in North Western Italy.

Spawning period lasted from 31 to 36 days (from 15 April to 22 May) with two peaks of oviposition (one in April and one in May), and an interruption ranging from 5 to 10 days. Number of egg masses laid was positively correlated with water temperature.

The mean number of eggs estimated in 100 egg masses was 59 ± 23 SD. 93% of observed eggs were fertile. The percentage of unfertile eggs was significantly greater in larger masses.

Larval period ranged from the first days of May to the middle of July, with an average duration of 66-67 days.

INTRODUCTION

Although the reproductive biology of European Anuran Amphibians has been and continues to be object of numerous studies, only a few publications refer to the tree frog *Hyla arborea* (Linnaeus, 1758). This species shows a wide distribution in Europe (ARNOLD & BURTON, 1978), and in the recent past it was rather common. Now *H. arborea* is a vulnerable species in Northern Europe (STUMPEL & HANEKAMP, 1986) as well as in North Western Italy, where it shows a localized distribution (PAVIGNANO & GIACOMA, 1986).

H. arborea is a rather terrestrial species; it only lives in aquatic habitats during the reproductive period. The aquatic habitat consists of ponds in grasslands and marshes. Extent of aquatic vegetation cover, surrounding terrestrial habitat, level of human interference are discriminant parameters for this species in choosing breeding sites (PAVIGNANO et al., 1989 a). Alteration of these ecological parameters is probably the cause of current sporadic distribution of species. Where the habitat is suitable, *H. arborea* occurs usually with a numerous breeding population (PAVIGNANO et al., 1989 b); this is probably due to the several reproductions during the breeding season. The reproductive period of *Hyla* is in fact a long one (DIAZ-PANIAGUA, 1986; GARTON & BRANDON, 1975; PERRIL & DANIEL, 1983); in Northern Italy two spawn periods of *H. arborea* have been observed (PAVIGNANO, 1990).

There is no specific work on reproductive biology of *H. arborea*; in this study I investigated some parameters of the breeding phenology and larval period of *H. arborea* in six

ponds in North Western Italy during the years 1988 and 1989. Particular emphasis was given to gathering precise information concerning the duration of spawning period, the number of eggs per clump, the number of fertile eggs, and duration of metamorphosis of tadpoles.

Materials and methods

The study area has been described in detail in a former paper (Pagignano, 1989) and consists of a series of temporary draining ditches in fields, of which six sites were populated by *H. arborea*.

Observations were made every day during the breeding period.

For the estimation of the total spawn, all the egg masses at each pond were counted during every visit and the newly laid egg masses were marked on maps. The total number of eggs per clump was calculated by direct count from randomly selected egg masses and in each clump the number of fertile eggs was determined by stereo-microscope.

Tadpole populations in the ponds were sampled once every two days. The larvae, captured for each sample with a dipnet in different location in the ponds, were counted and larval stages (according to Gosner, 1960) of each species was determined. Tadpoles were then released. A small number of eggs and tadpoles was fixed in 60 % ethanol for microscopical observation of exact larval stage, and then photographed.

Maximum and minimum water and air temperature was recorded daily by a maximum/minimum thermometer placed in each pond, together with general conditions (i.e. if it had rained or not, etc.). The local climate is classified as continental with rainfall maxima in the middle of April (mean = 113mm) and November (mean = 79 mm); minima occurs in January (mean = 32 mm) and July (mean = 79 mm) (Durio et al., 1983).

Results

In Northern Italy, *H. arborea* begins to spawn between the middle and end of April. During the two years of my observations the spawning season lasted 36 days in 1988 (from 15 April to 20 May) and 31 days in 1989 (from 22 April to 22 May). In the 1988 breeding season a total of 404 laid egg masses (mean = 67.33 ± 22.06 SD for each pond) was recorded, and 354 (mean = 59.00 ± 27.63 SD) in 1989. No oviposition occurred when minimum water temperature was below 8°C and air temperature below 6°C. During the spawning period average water temperature varied between 10-22°C. Number of egg masses laid was positively correlated with average water temperature ($r = 0.469$, $p < 0.005$). In both years there were two peaks of oviposition (fig. 1) : 15 to 21 April and 15 to 18 May for 1988 (in those periods 82% of egg masses were laid, 60% of which in April) ; 25 to 29 April and 13 to 17 May for 1989 (84% of egg masses, 59% of which in April). The maximum spawning activity took place in April. An interruption of oviposition for about ten days in 1988 (5 to 14 May) and five days in 1989 (9 to 13 May) was observed. The laid egg masses were observed either before or after rainy days; no significant correlation was recorded between the number of laid egg masses and rain.

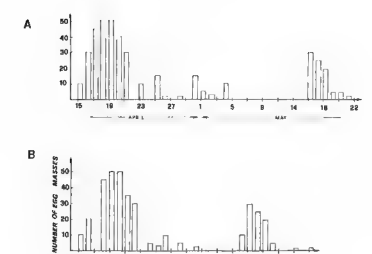


Fig. 1. – Number of egg masses per day laid in 1988 (a) and 1989 (b) spawning seasons.

The egg masses were laid in small groups, near the pond edges, at a depth of 5 to 10 cm, fixed on the vegetation. The shape of the egg masses was oval, the colour of the eggs was pale with grey animal hemisphere.

In the first days of October a further breeding activity was observed, revealing itself only by male calls. Males called from aquatic vegetation, but in a shorter and less frequent way than in April.

The number of eggs per mass determined by counting in 100 randomly selected egg masses is shown in Table I. The mean number of eggs per mass in the two years was 59 (± 23 SD) eggs. The difference in the mean number of eggs in the two years was not statistically significant (Student's $t = 1.75$, $p > 0.05$). 60% of egg masses included 60–100 eggs. Very small egg masses (20-30 eggs) were laid at the end of the spawning period and comprised 30% of total; there were also a few very large egg masses of more than 100 eggs comprising 5% of total. 93% of observed eggs were fertile; the percentage of unfertile eggs was significantly (Student's $t = 0.78$, $p < 0.05$) greater in large masses. In masses with 20-30 eggs, they were all fertile.

Table I. – Number of eggs per mass observed in 1988 and 1989 spawning seasons (*number of fertile eggs).

Year	N	Max	Min	M	SD
1988	50	125	20	57	28
		*120	20	54	27
1989	50	118	25	62	18
		*110	25	56	12
Total	100	125	20	59	23
		*120	20	55	20

Average larval period duration was of 67 days ± 1.02 SD in 1988 and 66 days ± 1.32 SD in 1989 (from the first days of May to the middle of July), from the first tadpoles leaving stage 25 (last embryonic stage according to GOSNER, 1960) until the last tadpoles completed their metamorphosis. Larval populations were composed of individuals at different development stages, because the various egg masses were laid in different times. There was a temporal overlap, lasting about 36 days, between tadpoles of different age and size classes. When the development of tadpoles from first ovipositions was between stage 34 and 40, there were tadpoles between stage 28 and 32; when the tadpoles from first ovipositions completed their metamorphosis, there were still tadpoles at stages 36 and 40.

DISCUSSION

The reproduction of Amphibians is usually related to weather factors, such as temperature and rainfall. *H. arborea* belongs to the type of Anurans which have a long spawning season; a first consequence of this is a relation with weather conditions. Although there were two oviposition peaks (about one month one after the other), this species seemed not to be particularly dependent on the rainfall, while no oviposition below 8°C water and 6°C air temperature was observed, and the observed oviposition was well correlated with temperature. Breeding activity not dependent on rain has also been observed in other species of *Hyla* (DIAZ-PANIAGUA, 1986).

A second consequence of the long spawning period was the temporal overlap of larval populations composed of individuals at different ages and sizes. The length of the larval period of *Hyla* is related to water temperature (DIAZ-PANIAGUA, 1986; STUMPEL & HANEKAMP, 1986); an increase of 5 – 8°C water temperature causes a shortening of about 10 days in *H. arborea*'s larval period (PAVIGNANO, 1990).

The variation in eggs number per mass shown by my results is in good agreement with LANZA (1983). The percentage of fertile eggs was related to the number of eggs per mass. Probably, as it happens in other Anuran species (HAAPENEN, 1982; SOFIANIDOU & KYRIAKOPOULOU-SKLAVOUNOU, 1983), the number of eggs laid by a female is dependent on its size. Quantitative information concerning the number and size of eggs from individual females, and relations with body size require further investigations.

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Cette liste fait suite à celles que nous avons déjà publiées (DUBOIS, 1988, 1989) pour les années 1982-1987, et a été préparée de la même manière. A ce sujet, il nous faut signaler ici une erreur qui s'est glissée dans la première de ces listes (DUBOIS, 1988) : le fascicule 4 du volume 1 d'*Alytes* a été publié en fait le 30 décembre 1982, et non pas le 31 décembre 1982.

Volume	Fascicule	Pages	Date figurant sur le fascicule	Date réelle de publication
7	2	45–76	Juin 1988	27 février 1989
7	3	77–124	Septembre 1988	11 juin 1989
7	4	125–168	Décembre 1988	3 novembre 1989
3-4	Index	i-xii	1984-1985	3 novembre 1989

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